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In: Silico analysis of intra: Specific variation of wasps venom protein phospholipase A1

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Abstract
In the present study, the Hymenopterans wasp *Vespa velutina (*Asian yellow-legged hornet), *Vespa affinis* (Lesser banded hornet) and *Vespula squamosa* (Southern yellow jacket) venom protein Phospholipase A1 sequences were selected for In-silico analysis. The venom protein sequence was retrieved from the UniProtKB protein database, using those sequences their physicochemical properties were analysed from the ProtParam tool. Secondary structural elements were predicted from the SOPMA tool. The 3D model was built using the SWISS-MODEL server. The final refined model was evaluated by using the PROCHECK, and Z-score. The venom protein Phospholipase A1, the basic molecular nature, composition and their biological properties were reported. ProtParam tool result shows that the protein belongs to the lipases family. The venom protein Phospholipase A1 is hydrolyzing the phospholipid at the 2-acyl-lysophospholipid position to the fatty acid. The most abundant amino acid was found as Leucine (28 residues) in Phospholipase A1 of Lesser banded hornet *V. affinis*, whereas the lowest was Tryptophan. It was observed that the random coil was predominant, followed by alpha-helix and extended strand in all the three wasp venom protein Phospholipase A1.The predicted 3D structure will support the understanding of the structure of the Phospholipase A1 in wasp species.

Keywords: hymenoptera, wasp, hornet, venom, and structure prediction

1. Introduction

The Vespidae is a cosmopolitan family of order Hymenoptera popularly called as wasps. The wasp *Vespa affinis* are insects in order Hymenoptera, and are commonly known as The banded tiger wasps in Southeast Asia[1]. They are harmful to human because of their nest is, widespread on trees or under eaves of houses [1]. The *Vespula squamosa* is distinctive in being either a free-living or a temporary, facultative parasite of other Vespula [2]**.**

The *Vespula squamosa*, the southern yellow jacket, is a social wasp, this species depends on insects and animal carcasses for their energy need. It does not produce honey. *V. squamosa* is an important nuisance species because of its preference for disturbed areas, resulting in frequent contact with humans [3]. The *Vespa velutina* a widespread and invasive species uses an alarm pheromone, produced by the sting gland, and increasing quantities of sting gland extract increased aggressive attacks. *V. velutina* can be dangerous for man after encountering multiple stings [4]**.** Their venom was used for defending themselves [1].

Stinging of the Hymenoptera insects, such as bees, wasps and ants may induce allergic reactions and, occasionally, fatal anaphylaxis. Hymenoptera venoms are complex mixtures of biochemically and medically active components such as biogenic amines, proteins and peptides.. The major part of venom allergens is Phospholipase A1 [5]**.**

The molecular information about detrimental or toxic factors in wasp venom is extremely lacking. Most animals with multiple wasp stings suffer from multiple organ dysfunctions elicited by toxic reactions rather than an anaphylactic reaction, which means that the wasp venom toxicity can be attributed to haemolytic, myotoxic, neurotoxic, vasodilatory, nephrotoxic and hepatotoxic enzymes [1-5].

The composition of vespid venoms has been subject to little study, since the production of venoms by the social wasps is very minimal and there is little availability of vespid venoms as raw materials. Vespinae venoms contain many deferent components such as phospholipases A and B, hyaluronidases, acid phosphatases, proteases and nucleotidases [6].

Stinging of the Hymenoptera wasps may induce allergic reactions and, occasionally, fatal anaphylaxis. Clinical manifestations of both local and systemic reactions such as redness site bite area, pain, swelling, and headache. The major proteins found in this *V.affinis* are phospholipase, antigen 5, di-peptidyl peptidase and hyaluronidase. Phospholipase A1 is one of the enzymes in phospholipase family that hydrolyzes phospholipid at the 2-acyl-lysophospholipid position to fatty acid [7]. Vespidae venom consists of complex mixtures of enzymes, proteins, peptides and small molecules accountable for many of the non-allergic and mild allergic reactions – such as local pain, inflammation and itching–as well as moderate and serious allergic reactions–such as anaphylaxis, and delayed hypersensitivity–including systemic toxic reactions, coagulopathy, acute renal failure and hepatotoxicity. Hyaluronidase and phospholipase A2, which are known to be the main components of wasp venom, along with other enzymes, including zinc-metallopeptidases [8].

Phospholipases are the most common enzymes in venoms. Phospholipase A2 known to be about 12% of the dry weight and it is the major antigen in bee venom. Phospholipases are so universal and play a functional role in receptor-mediated reactions as well as causing cell lysis [9].

Hymenoptera venoms are complex mixtures of biochemically and medically active components such as biogenic amines, proteins and peptides. The composition of vespid venoms has been subject to little study, since the production of venoms by the social wasps is very minimal and there is little availability of vespid venoms as raw materials. Vespinae venoms contain many deferent components such as phospholipases A and B, hyaluronidases, acid phosphatases, proteases and nucleotidases [4]. Comparative modelling predicts the 3-D structure of a given protein sequence (target) based primarily on its alignment to one or more proteins of known structure (templates) Functional characterization of a protein sequence is one of the most frequent problems in biology. In the absence of an experimentally determined structure, comparative or homology modelling can sometimes provide a useful 3-D model for a protein that is related to at least one known protein structure [10].

The aim of this present study was intra-specific variation of in primary, secondary structure, and three-dimensional (3-D) modelling of venom protein Phospholipase A1 were performed with various bioinformatics tools.

2. Materials and Methods

2.1 Sequences

The Hymenopterans venom protein Phospholipase A1 sequences of three wasp's species such as *Vespa velutina (*Asian yellow-legged hornet), *Vespa affinis* (Lesser banded hornet) and *Vespula squamosa* (Southern yellow jacket) sequences were retrieved from UniProtKB protein database. UniProtKB is public protein database which contains the amino acid sequences of proteins. The protein sequences of Phospholipase A1 were retrieved and saved in FASTA file format with their accession IDs.

2.2 Physicochemical Analysis

The physicochemical analysis was performed by using ProtParam tool. ProtParam is a tool that allows the computation of various physical and chemical parameters for a given protein stored in Swiss-Prot or TrEMBL or for a user entered protein sequence.

The molecular mass and isoelectric points were computed using the Compute pI/MW tool of ExPASy Bioinformatics (http://web.expasy.org/computepi/). The Computed para meters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY).

2.3 Domain Analysis

The molecular domains of venom protein Phospholipase A1 of wasp species were analysed using the Pfam database. Pfam server (http://www.sanger.ac.uk/software /pfam/ search. html) was used for domain analysis [14].

2.4 Secondary Structure Prediction:

The secondary structures of venom protein Phospholipase A1were predicted by using SOPMA (Self- Optimized Prediction Method with Alignment). Secondary structure prediction was performed by using SOPMA [15] server (http://npsapbil.ibcp.fr/). Sub-cellular localization was predicted by using CELLO v.2.5 [15-17]. 1.1 server (http:// www.cbs.dtu.dk). Motif Scan [17-18]. Server (http:// myhits.isb-sib.ch/cgibin/motif_scan) was used to identify known motifs in the sequence.

2.5 Homology Modeling and model Validation

The three-dimensional structures of wasps venom protein Phospholipase A1 were modelled using SWISS-MODEL server. The three-dimensional models were created using the SWISS-MODEL program, the automated protein homology modeling template at ExPASY (Switzerland) and a template search with the Alignment Mode program from the protein database (http://swissmodel.expasy.org/). The SWISS-MODEL is a structural bioinformatics web-server dedicated to the homology modelling of protein 3D structures. After modelling, the quality and validation of the model was evaluated by several structure assessment methods, containing Z-Score by using QMEAN [19]. SAVES V 5.0 server for Ramachandran plot analysis [20].

3. Results and Discussion

The wasps *Vespa velutina (*Asian yellow-legged hornet), *Vespa affinis* (Lesser banded hornet) and *Vespula squamosa* (Southern yellow jacket) venom protein Phospholipase A1 sequences were retrieved from the UniProtKB database and the sequence was saved in the FASTA file format. The UniProtKB IDs, sequence length ranges from 304-334 is presented in the table1.

Table 1: The wasp venom protein Phospholipase A1 sequences.

Species Names	*Vespa affinis*	*Vespula squamosa*	*Vespa velutina*
Common name	Lesser banded hornet	Southern yellow jacket wasp	Asian yellow-legged hornet
Protein name	Phospholipase A1	Phospholipase A1	Phospholipase A1
UniProtKB ID	P0DMB4 (PA11_VESAF)	P0CH86 (PA1_VESSQ)	C0HLL3 (PA1_VESVE)
Length	334	294	304
Molecular weight	37318.00	32488.30	33957.16

The physicochemical analyses of the predicted venom protein Phospholipase A1 were performed using Protparam tool and results were shown in Table 2. This venom protein Phos pholipase had 304 to334 amino acids with a molecular weight ranges from 32488.30 to 37318.00 Daltons and *pI* 8.70 to 9.08 its indicates that this protein is accepted as basic. Table 2.

Table 2: The physicochemical Properties of wasps venom protein Phospholipase A1.

Parameters	*Vespa affinis*	*Vespula squamosa*	*Vespa velutina*	Explanation
pI	8.70	8.96	9.08	The protein is accepted as basic
Total number of negatively charged residues (Asp + Glu)	25	27	24	Total numbers of negatively charged residues are lesser than the total number of positively charged residues.

Total number of positively charged residues (Arg + Lys)	33	37	37	Total numbers of positively charged residues are higher than the total number of negatively charged residues.
The instability index (II)	37.82	37.53	38.17	This classifies the protein as stable
Aliphatic index	82.90	83.16	81.12	Globular protein
Grand average of hydropathicity (GRAVY)	-0.146	-0.162	-0.260	The negative score indicates this protein is hydrophilic in nature

The most abundant amino acid was found as Leucine (28 residues) in Phospholipase A1 of Lesser banded hornet *V. affinis*, whereas the lowest was Tryptophan (1 residue). The total number of positively charged residues (Arg + Lys, 33) was found higher than the total number of negatively charged residues (Asp + Glu, 33).The Asian yellow-legged hornet *V. velutina* venom protein Phospholipase A1 shows that, the most abundant amino acid was found as Lysine (26 residues), whereas the lowest was Tryptophan (1residue). The total number of positively charged residues (Arg + Lys, 37) was found higher than the total number of negatively charged residues (Asp + Glu, 24). The Southern yellow jacket *V. squamosa* venom protein Phospholipase A1 recorded that the most abundant amino acid was found as Valine (26 residues) and Serine (26 residues), whereas the lowest was Tryptophan (1residue). The total number of positively charged residues (Arg + Lys, 37) was found higher than the total number of negatively charged residues (Asp + Glu, 27) (Table2). Intracellular proteins have a lower number of cysteine residues, but also higher numbers of aliphatic and charged amino acid residues [21]. This data is in agreement with our finding that the highest numbers of amino acids residues were Leucine and Lysine while the lowest one was Tryptophan. The instability (II) and aliphatic index revealed that this protein may be stable and globular protein. Negative GRAVY value shows that this protein is accepted as a hydrophilic character (Table 2). It is accepted that extracellular proteins include more disulphide bridges and Cysteine residues [22]. The predicting of sub-cellular localization of unknown proteins contributes to the understanding of their functions [23]. It was performed using CELLO v.2.5 and venom protein Phospholipase A1 was localized in extracellular matrix.

The secondary structures of the venom protein Phospholipase A1 were predicted using the SOPMA server. The secondary structure elements alpha helix, beta-turn, extended strand and random coils were calculated and presented in Table 3.

Table 3: Secondary structure analysis of wasp's venom protein Phospholipase A1using SOPMA server

Species name	**Vespa affinis**		**Vespula squamosa**		**Vespa velutina**	
Parameters	**Amino acids**	**Amino acids %**	**Amino acids**	**Amino acids %**	**Amino acids**	**Amino acids %**
Alpha helix (Hh)	74	22.16%	74	25.17%	61	20.07%
helix (Gg)	0	0.00%	0	0.00%	0	0.00%
Pi helix (Ii)	0	0.00%	0	0.00%	0	0.00%
Beta bridge (Bb)	0	0.00%	0	0.00%	0	0.00%
Extended strand (Ee)	75	22.46%	62	21.09%	62	20.39%
Beta turn (Tt)	17	5.09%	15	5.10%	16	5.26%
Bend region (Ss)	0	0.00%	0	0.00%	0	0.00%
Random coil (Cc)	168	50.30%	143	48.64%	165	54.28%
Ambiguous states	0	0.00%	0	0.00%	0	0.00%
Other states	0	0.00%	0	0.00%	0	0.00%

It was observed that the random coil was predominant, followed by alpha helix and extended strand in all the three wasp venom protein Phospholipase A1. Also, beta-turn was found as average of 5%. (Table 3). Random coils have important functions in proteins for flexibility and conformational changes such as enzymatic turnover[24]. Our findings could be related with the enzymatic function of protein. The domain analysis was conducted using the Pfam database and the venom protein Phospholipase A1 belongs to the Lipase family. Phospholipases are so universal and play a functional role in receptor-mediated reactions as well as causing cell lysis. This data support that our protein may play a role as an enzyme in hydrolysis reactions (Figure 1). Phospholipase A1 (PLA1) is one of the enzymes in phospholipase family that hydrolyzes phospholipid at the 2-acyl-lysophospholipid position to fatty acid[7].

Fig 1: Domain Structure of wasp's venom protein Phospholipase A1 is Lipase family.

The Motif scan tool was used to determine different motifs. The seven type's motifs were observed and the highest number of the motif was Myristoylation site, and Protein kinase C phosphorylation site. CK2-Phosphorylation site, ASN- Glycosylation site, Amidation, CAMP- Phospho -site and Lipase (Table 4).

Table 4: The motifs of predicted wasp's venom protein Phospholipase A1 by Motif scan tool

Species name	***Vespa affinis***	***Vespula squamosa***	***Vespa velutina***
Motif information	**No. of sites (sequences)**	**No. of sites (sequences)**	**No. of sites (sequences)**
ASN-Glycosylation site	-	2	2
CK2-Phosphorylation site	1	4	2
Myristoylation site	6	4	8
Protein kinase C phosphorylation site	5	4	4
Amidation	1	-	1
CAMP- Phospho -site	1	-	
Lipase	1	1	1

The phosphorylation of a protein can affect functions and activities of proteins, including intrinsic biological activity, half-life, subcellular location, and bind with other proteins[25].
Myristoylation is Post-translational Protein modification observed In plants, animals, fungi, and viruses; it is performed by attached myristic acid in proteins.
Myristoylation can affect the conformational stability of proteins by interaction with membranes or the hydrophobic domains of other proteins[26].

Table 5: Homology modelling wasp's venom protein Phospholipase A1 and model validation

Species Name	*Vespa affinis*	*Vespula squamosa*	*Vespa velutina*
Template and organism name	**4qnn.1.A *Vespa basalis* venom Crystal Structure of phospholipase A 1**	**4qnn.1.A *Vespa basalis* venom Crystal Structure of phospholipase A 1**	**4qnn.1.A *Vespa basalis* venom Crystal Structure of phospholipase A 1**
Template identity	92.31%	69.10%	74.07%
Ramchandran plot- (Residues in most favoured regions)	88.7%	87.9%	85.3%
Ramchandran plot- (Residues in additional allowed regions)	9.6%	10.2%	10.9%
Qmean-Value	-0.53	-1.90	-2.17

The Swiss-Model homology modeling program was used for the predicting three- dimensional structure of the Hyaluronidase (HYases) (Figure 3-5). Uniprot Id/PDB 4qnn.1.A (*Vespa basalis* venom Crystal Structure of phospholipase A 1) was selected as a template with *Vespa affinis (*92.31%), *Vespula squamosa* (69.10%), and *Vespa velutina* (74.07%) sequence identity to query sequence (Figure 2-4).
After model building, the structure was validated through energy minimization with Z-Score by using the Qmean server, and Ramachandran plot analysis. The Z-score is used to estimate the quality of model using structured solved proteins as references[27]. Qmean score for this protein is -0.53 to -2.17 were recorded. The overall quality factor was found as 95.0 which are satisfactory. Ramachandran plot analysis of wasp venom protein Phospholipase A1showed that the residues in most favoured regions are 88.7%, 87.9%and 85.3%.03% respectively. The residues in additional allowed regions is 9.6%, 10.2% and 10.9% indicating that the model was of reliable and good quality (Table 4).

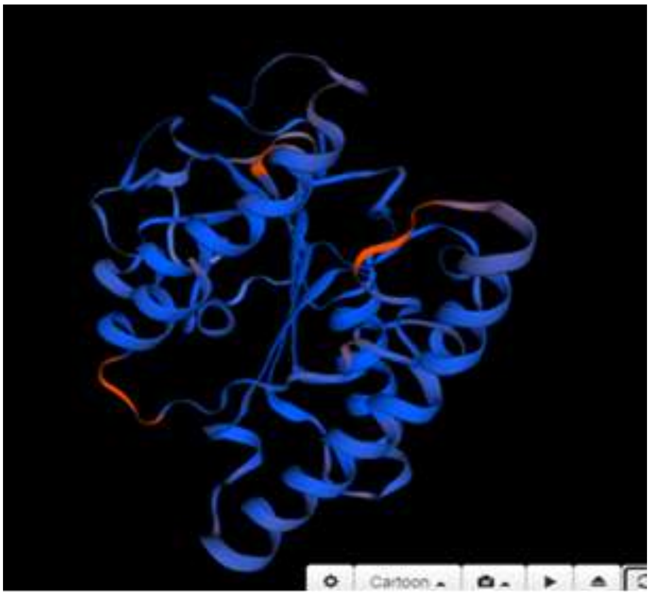

Fig 3: Three dimensional model of wasp *Vespula squamosa* venom protein Phospholipase A1

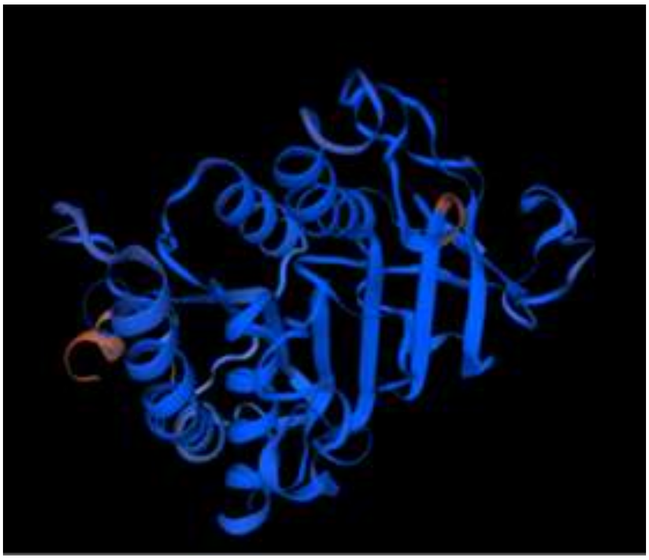

Fig 2: Three Dimensional Model of wasp *Vespa affinis* venom protein Phospholipase A1

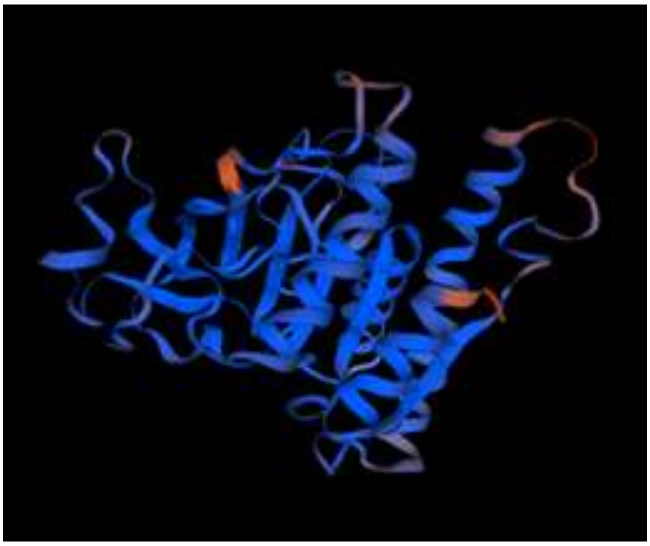

Fig 4: Three dimensional model of wasp *Vespa velutina* venom protein Phospholipase A1

The increasing knowledge of the exact composition of hymenoptera venoms created added value for accurate diagnosis of venom allergy. Moreover, it turns out that the understanding of all allergen components, not only in the venom, but also in therapeutic extracts used for specific immunotherapy, might influence the outcome of the therapy [28-29].

4. Conclusion

The wasp *Vespa velutina (*Asian yellow-legged hornet), *Vespa affinis* (Lesser banded hornet) and *Vespula squamosa* (Southern yellow jacket) venom protein Phospholipase A1 sequences were selected for In-silico analysis. In the present study, sequence and structural insight of venom protein Phospholipase A1 their basic molecular nature with understanding and composition and their biological properties were reported. The venom protein sequence was retrieved from UniProtKB protein database, using those sequences their physicochemical properties were analysed from the ProtParam tool. Its show that the protein is lipases family. Phospholipase A1 is one of the enzymes in phospholipase family that hydrolyzes phospholipid at the 2-acyl-lysophospholipid position to the fatty acid. Secondary structural elements were predicted from the SOPMA tool. The 3D model was built using the SWISS-MODEL server. The final refined model was evaluated by using the PROCHECK. The most abundant amino acid was found as Leucine (28 residues) in Phospholipase A1 of Lesser banded hornet *V. affinis*, whereas the lowest was Tryptophan. It was observed that the random coil was predominant, followed by alpha-helix and extended strand in all the three wasp venom protein Phospholipase A1. The predicted 3D structure will support the understanding of the structure of the Phospholipase A1 in wasp species.

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Conflict of interest

The authors declare that they have no conflict of interest.

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